women) who were clinically diagnosed as having infectious disease were studied. The median age was 68, with an interguartile range of 52, 76 years. There were 95 patients with a respiratory tract infection (pneumonia, bronchitis, tonsillitis or a common cold), 10 with a biliary tract infection (cholecystitis and cholangitis), 33 with a gastrointestinal infection (acute enteritis, appendicitis or other enterocolitis), 8 with a urinary tract infection (pyelonephritis, pyelitis or cystitis), and 7 with other infections (parotitis, vaginitis, rickettsiosis, herpes zoster, decubitus and others). All were diagnosed at an outpatient clinic or on admission to our hospital or other hospitals. Most of the patients were at the early to peak phase of infection. Patients having complications with disseminated intravascular coagulation (DIC), diabetes mellitus, cerebrovascular disease, cancer, renal dysfunction (serum creatinine $>110\ \mu molL^{-1}$), or patients undergoing anticoagulant therapy were excluded. Thirty-seven age-matched, asymptomatic healthy volunteers (14 men and 23 women) with no diseases and no elevations of C-reactive protein (CRP) served as controls.

Collection of blood samples

Venous blood was collected into siliconized tubes containing 1/10 volume of $0.129\ molL^{-1}$ trisodium citrate using a 10-ml syringe with a 21-gauge needle, and was centrifuged at 1,500g for 15 min at 4℃. Serum samples for the determinaton of fibrin degradation products (FDP) were prepared by collecting whole blood into glass tubes containing thrombin, reptilase, aprotinin and calcium. Assays were performed on fresh plasma and serum samples or samples stored at −70℃ as follows.

Assay methods

Plasma TM was measured by an enzyme-linked immunosorbent assay (ELISA) (Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan)[10]. White blood cells (WBC), granulocytes, monocytes, and lymphocytes were counted by standard methods. Fibrinogen was measured by the thrombin time method. FDP was measured by a semiquantitative latex agglutination method (Teikokuzoki, Tokyo). Cross-linked fibrin degradation products (D-dimer) were measured by a latex photometric immunoassay (Dia-Iatron Laboratories Inc., Tokyo). Antithrombin III (AT III) was measured by a chromogenic substrate-based technique (Dia-Iatron). Thrombin-antithrombin III complex (TAT) and plasmin-α2-plasmin inhibitor complex (PIC) were measured by ELISA (Teijin Ltd., Tokyo). Protein C and von Willebrand factor antigen (vWf: Ag) were measured by ELISA (Boehringer Mannheim, Tokyo). Polymorphonuclear neutrophil elastase (PMN elastase) was measured as granulocyte elastase-α1-proteinase inhibitor complex by ELISA (Diagnostica Merck, Darmstadt, Germany). CRP was measured by a latex agglutination method (Denkaseiken, Tokyo). Serum creatinine was measured by an enzymatic method (Denkaseiken). The normal values are: fibrinogen $1.78\text{-}3.18\ gL^{-1}$, FDP$<2500\ \mu gL^{-1}$, D-dimer$\leqq 460\ \mu gL^{-1}$, ATIII 96-160%, TAT$\leqq 0.35\ \mu gL^{-1}$, PIC $400\text{-}1200\ \mu gL^{-1}$, protein C 69-157 % and vWf: Ag 52-292 %.

Statistical analysis

Medical parameters including skewed data are presented as median and the interguartile range in parenthesis. The significance of deifferences between two groups was tested by Mann-Whitney's U-test. Differences between groups were considered significant at a P value of <0.05. Relationships among parameters were analyzed by regression analysis using the least squares method, and correlation coefficients (r) were calculated. Multiple regression analysis with stepwise method was also done.

RESULTS

Levels of plasma TM and other parameters

In the 153 patients with infections diseases, the plasma TM concentration was between 11000 and 58600 UL^{-1}, and the median value 21400 (17500, 26700) UL^{-1} was significantly higher than that of the age-matched normal subjects 18200 (15100, 21300) UL^{-1} ($P<0.001$) (Table 1 and Fig. 1). Twenty-nine percent of the patients showed an elevated TM value, i.e., > mean+2SD of normal values. As shown in Table 1, the plasma levels of TAT, PIC, D-dimer, fibrinogen, vWf: Ag and PMN elastase were significantly higher in the patients than in the normal subjects ($P<0.001$). The plasma levels of AT III and protein C were significantly lower in the patients than in the normal subjects ($P<0.001$). The CRP and WBC levels were also elevated in the patients (Table 1).

Relationships between plasma TM and other parameters

The relationships between plasma TM and the other parameters are shown in Table 2. Among the various coagulation and fibrinolytic parameters, plasma TM

Table 1. Plasma levels of various parameters in patients with infectious disease and normal subjects

Parameter	Control(n=37)	Patients(n=153)	(Ranges of patients)
Age (y)	67(63, 70)	68(52, 76)	(12 ~89)
TM (UL^{-1})	18200(15100, 21300)	21400(17500, 26700)***	(11000~58600)
TAT (μgL^{-1})	1.1(0.8, 2.0)	4.0(2.0, 7.5)***	(0.8~47)
PIC (μgL^{-1})	800(600, 900)	1200(800, 1800)***	(0~500)
FDP (μgL^{-1})	2500(2500, 2500)	2500(2500, 2500)	(2500~40000)
D-dimer (μgL^{-1})	40(10, 210)	940(540, 2170)***	(10~18410)
ATIII (%)	126(118, 141)	109(93, 123)***	(41~163)
Protein C (%)	110(103, 122)	83(62, 94)***	(29~145)
Fibrinogen (gL^{-1})	2.46(2.23, 2.69)	3.51(2.97, 4.42)***	(1.76~6.41)
vWf:Ag (%)	155(125, 200)	240(165, 320)***	(39~740)
PMN elastase (μgL^{-1})	107(84, 127)	194(138, 311)***	(0~2264)
CRP (gL^{-1})	<0.005	0.042(0.007, 0.0138)	(0~0.0284)
Creatinine ($\mu molL^{-1}$)	<110	62(53, 80)	(27~110)
WBC (10^9L^{-1})	3.9~8.9	7.3(5.5, 10.3)	(2.7~23.7)
Granulocytes (10^9L^{-1})	——	5.3(3.4, 8.5)	(1.5~22.3)
Monocytes (10^9L^{-1})	——	0.4(0.3, 0.6)	(0~2.3)
Lymphocytes (10^9L^{-1})	——	1.3(0.8, 1.8)	(0.2~4.8)

Median values (interquartile range P25, P75) are shown.
Observed ranges of patients are shown as the Ranges of patients in parentheses.
***P<0.001 (compared with normal subjects)

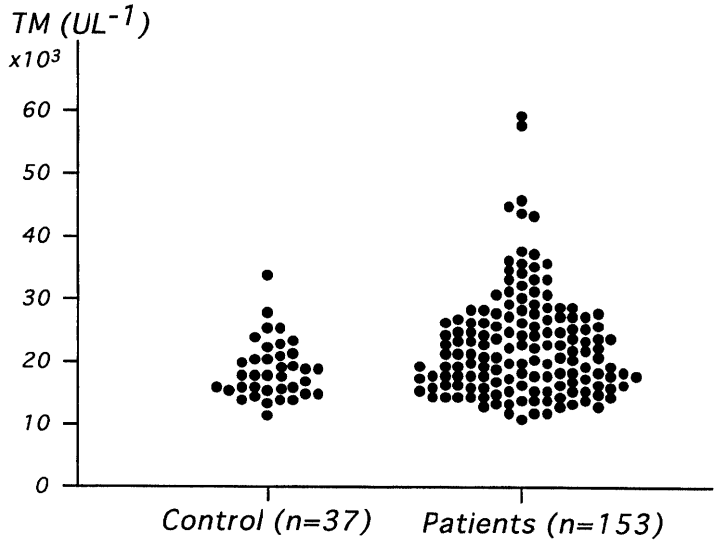

Fig. 1. A scatterplot diagram of TM values in the control and patient groups.

correlated positively with PIC, FDP, D-dimer and vWf: Ag, and negatively with protein C. In addition, the plasma TM correlated with the granulocyte counts, but not with the monocyte or lymphocyte counts. PMN elastase correlated positively with the WBC and granulocyte counts, CRP, fibrinogen and vWf: Ag, and negatively with AT III and protein C. However, the correlation between TM and PMN

elastase was not significant in the patients as a whole ($r=0.122$, $P<0.2$) (Table 2). Thirty-four percent of the patients with elevated PMN elastase had elevated TM, and 22% of the patients with normal PMN elastase showed an elevation of TM. Plasma TM was also correlated with serum creatinine ($P<0.05$) at the level $\leqq 110\ \mu molL^{-1}$. Levels of D-dimer, protein C and creatinine were significantly related with TM level in multiple regression analysis with a stepwise method.

Table 2. Correlations between plasma TM and other parameters in the patients with infectious diseases

Parameter	r	r^2	P value
Age	0.110	0.012	NS
TAT	0.121	0.015	NS
PIC	0.193	0.037	$P<0.05$
FDP	0.215	0.046	$P<0.01$
D-dimer	0.387	0.150	$P<0.001$
ATIII	−0.149	0.022	NS
Protein C	−0.309	0.095	$P<0.001$
Fibrinogen	−0.028	0.001	NS
vWf:Ag	0.291	0.085	$P<0.001$
PMN elastase	0.122	0.015	NS
CRP	0.137	0.019	NS
Creatinine	0.189	0.036	$P<0.05$
WBC	0.133	0.018	NS
Granulocytes	0.231	0.053	$P<0.01$
Monocytes	−0.099	0.010	NS
Lymphocytes	−0.151	0.023	NS

Analysis according to CRP

The patients were divided into two groups according to the level of CRP. As shown in Table 3, no difference was found in the TM level between the mild infection (CRP<5.0 mg/dl) group (n=75) and severe infection (CRP≧5.0 mg/dl) group (n=73), although the groups' PMN elastase, WBC count, granulocyte count and CRP differed significantly.

Analysis according to age

We then subdivided the patients into two groups according to their ages to identify any difference in the degree of endothelial cell damage between older patients (age>60 years) and younger patients (age≦60 years) for convenience, and analyzed. The TM level, granulocyte counts and vWf: Ag were significantly higher in the older patients than in the younger patients (data: not shown). In the older patients, TM correlated positively with WBC and granulocyte counts, CRP, PIC, FDP, D-dimer and vWf: Ag. In the younger patients, TM correlated only with the monocyte count (Table 4).

DISCUSSION

An assay of soluble TM in human plasma and urine by ELISA was recently established[10] and has been used as a marker for the injury of endothelial cells[6]. It is known that the plasma level of TM is elevated in patients with systemic lupus erythematosus[11], diabetes mellitus[12,13], adult respiratory distress syndrome[14], thrombotic disease[12,14] and DIC[12,14]. Although patients with mild infections were included in

Table 3. Plasma levels of each parameter in patient groups divided according to the level of C-reactive protein (CRP)

Parameter	CRP<5.0 mg/dl (n=75)	CRP≧5.0 mg/dl (n=73)	P value
TM (UmL^{-1})	22.0±8.2	24.4±8.0	NS
PMN elastase (μgL^{-1})	179±148	427±394	$P<0.001$
WBC (μL^{-1})	6514±2450	10457±4331	$P<0.001$
Granulocytes (μL^{-1})	4663±2984	8684±4198	$P<0.001$
Monocytes (μL^{-1})	452±235	528±371	NS
Lymphocytes (μL^{-1})	1492±780	1188±679	$P<0.05$
CRP ($mgdL^{-1}$)	1.19±1.22	14.50±6.26	$P<0.001$
Age (y)	60±21	63±19	NS

Table 4. Correlation coefficients between plasma TM and other parameters in the older and younger patient groups

Parameter	Age≦60 y (n=50)	Age>60 y (n=103)
PIC	r=0.103	0.228*
FDP	0.198	0.211*
D-dimer	0.157	0.482***
vWf:Ag	0.159	0.273**
PMN elastase	0.080	0.147
CRP	−0.104	0.202*
WBC	−0.204	0.261**
Granulocytes	−0.111	0.334***
Monocytes	−0.348*	−0.0003
Lymphocytes	−0.257	−0.074

*P<0.05, **P<0.01, ***P<0.001.

the present study, and elevation of plasma TM was found. These patients showed signs of activation of coagulation and fibrinolysis: elevated TAT, PIC and D-dimer (Table 1). Significant correlations between the TM level and PIC, FDP and D-dimer were found (Table 2). In addition, the plasma TM correlated with vWf: Ag, another marker of endothelial cell injury. These findings suggest that endothelial cell damage is common in patients with infectious diseases although what is not obviously elevated, and could be a result of the activation of blood coagulation and fibrinolysis.

It has been reported that neutrophils mediate endothelial cell injury *in vitro*[15,16], and that this injury is inhibited by granulocyte elastase inhibitors but not by catalase or superoxide dismutase[15-17]. In addition, granulocyte elastase leads to hemostatic disorders in severe infections[18] and septic shock[19]. Decreased TM expression in various infected endothelial cells has also been reported[20,21]. In the present study, the TM levels did not significantly correlate with the level of PMN elastase and CRP, and the elevation of the plasma TM level was not so high in the high CRP group (Table 2 and 3), suggesting that some factors other than PMN elastase may affect the liberation of TM from endothelial cells. Recently, some reports have shown the importance of oxygen radicals in endothelial injury[22,23]. Although the elevation of the plasma TM level may not be remarkably high in a patient at the early phase of infection as in this study, endothelial cell damage may still be present, and careful hemostatic observations are necessary to prevent or treat thrombosis and organ disorder during the course of infection.

Regarding the patients' ages, the TM levels, granulocyte counts and vWf: Ag were significantly higher in the older patients although there was no difference in CRP between the two age groups. Only in the older group did the plasma TM correlate with the WBC, granulocyte counts, and other parameters (PIC, FDP, D-dimer, and vWf: Ag) (Table 4). These findings suggest that increased granulocytes easily cause damage to endothelial cells and lead to a procoagulant state in the older patient group compared with the younger patient group.

Even with the results of the present study, it is difficult to identify the key factors which elevate the plasma TM level in association with the activation of blood coagulation and fibrinolysis in patients with various infectious diseases. If we could analyse definite bacterial infection cases only and another relationship between TM and PMN elastase could be found, other factors such as other cellular enzymes, cytokines, and the condition of endothelial cells in the infectious state remain to be studied in addition to PMN elastase for causing the elevation of TM in this syudy.

Acknowledgments. This study was supported in part by a Research Grant for Intractable Diseases from the Ministry of Health and Welfare of Japan (to H. T.).

REFERENCES

1) Esmon NL: Thrombomodulin. *Semin Thromb Hemost* **13**: 454-463, 1987.
2) Jakubowski HV, Kline MD, Owen WG: The effect of bovine thrombomodulin on the specificity of bovine thrombin. *J Biol Chem* **261**: 3876-3882, 1986.
3) Esmon CT: The roles of protein C and thrombomodulin in the regulation of blood coagulation. *J Biol Chem* **264**: 4743-4746, 1989.
4) Dittman WA, Majerus PW: Structure and function of thrombomodulin: A natural anticoagulant. *Blood* **75**: 329-336, 1990.
5) Suzuki K, Kusumoto H, Deyashiki Y, Nishioka J, Maruyama I, Zushi M, Kawahara S, Honda G, Yamamoto S, Horiguchi S: Structure and expression of human thrombomodulin, a thrombin receptor on endothelium acting as a cofactor for protein C activation. *EMBO J* **6**: 1891-1897, 1987.
6) Ishii H, Uchiyama H, Kazama M: Soluble thrombomodulin antigen in conditioned medium is increa-

sed by damage of endothelial cells. *Thromb Haemost* **65**: 618-623, 1991.
7) Takahashi H, Tatewaki W, Wada K, Hanano M, Shibata A: Thrombin vs. plasmin generation in disseminated intravascular coagulation associated with various underlying disorders. *Am J Hematol* **33**: 90-95, 1990.
8) Philippe J, Offner F, Declerck PJ, Leroux-Roels G, Vogelaers D, Baele G, Collen D: Fibrinolysis and coagulation in patients with infectious disease and sepsis. *Thromb Haemost* **65**: 291-295, 1991.
9) Seki Y, Takahashi H, Niwano H, Wada K, Takakuwa E, Shibata A: Activation of blood coagulation and fibrinolysis in patients with infectious disease. *Jpn J Thromb Hemost* **3**: 412-419, 1992.
10) Ishii H, Nakano M, Tsubouchi J, Isikawa T, Uchiyama H, Hiraishi S, Tahara C, Miyajima Y, Kazama M: Establishment of enzyme immunoassay of human thrombomodulin in plasma and urine using monoclonal antibodies. *Thromb Haemost* **63**: 157-162, 1990.
11) Kodama S, Uchijima E, Nagai M, Mikawatani K, Hayashi T, Suzuki K: One-step sandwich enzyme immunoassay for soluble human thrombomodulin using monoclonal antibodies. *Clin Chim Acta* **192**: 191-200, 1990.
12) Takahashi H, Ito S, Hanano M, Wada K, Niwano H, Seki Y, Shibata A: Circulating thrombomodulin as a novel endothelial cell marker: Comparision of its behavior with von Willebrand factor and tissue-type plasminogen activator. *Am J Hematol* **41**: 32-39, 1992.
13) Iwashima Y, Sato T, Watanabe K, Oshima E, Hiraishi S, Ishii H, Kazama M, Makino I: Elevation of plasma thrombomodulin level in diabetic patients with early diabetic nephropathy. *Diabetes* **39**: 983-988, 1990.
14) Takano S, Kimura S, Ohdama S, Aoki N: Plasma thrombomodulin in health and diseases. *Blood* **76**: 2024-2029, 1990.
15) HarIan JM, Killen PD, Harker LA, Striker GE: Neutrophil-mediated endothelial injury in vitro. Mechanism of cell detachment. *J Clin Invest* **68**: 1394-1403, 1981.
16) Smedly LA, Tonnesen MG, Sandhaus RA, Haslett C, Guthrie LA, Johnson Jr. RB, Henson PM, Worthen GS: Neutrophil-mediated injury to endothlial cells. Enhancement by endotoxin and essential role of neutrophil elastase. *J Clin Invest* **77**: 1233-1243, 1986.
17) Weiss SJ, Regiani S: Neutrophils degrade subendothelial matrices in the presence of alpha-l-proteinase inhibitor. Cooperative use of lysosomal proteinase and oxygen metabolites. *J Clin Invest* **73**: 1297-1303, 1984.
18) Seitz R, Wolf M, Egbring R, Radtke KP, Liesenfeld A, Pittner P, Haveman K: Participation and interactions of neutrophil elastase in haemostatic disorders of patients with severe infections. *Eur J Haematol* **38**: 231-240, 1987.
19) Seitz R, Wolf M, Egbring R, Havemann K: The disturbance of hemostasis in septic shock: role of neutrophil elastase and thrombin, effects of antithrombin III and plasma substitution. *Eur J Haematol* **43**: 22-28, 1989.
20) Teysseire N, Arnoux D, Geoge F, Sampol J, Raoult D: von willebrand factor release and thrombomodulin and tissue factor expression in rickettsia cororii infected endothelial cells. *Infect Immun* **60**: 4388-4393, 1992.
21) Key NS, Vercellotti GM, Winkelmann JC, Moldow CF, Goodman JL, Esmon NL, Esmon CT, Jacob HS: Infection of vascular endothelial cells with herpes simplex virus enhances tissue factor activity and reduces thromobomodulin expression. *Proc Natl Acad Sci* **87**: 7095-7099, 1990.
22) Hardy MM, Flickinger AG, Riley DP, Weiss RH, Ryan US: Superoxide dismutase mimetics inhibit neutrophil-mediated human aortic endothelial cell injury in vitro. *J Biol Chem* **269**: 18535-18540, 1994.
23) Varani J, Ward PA: Mechanisms of neutrophil-dependent and neutrophil-independent endothelial cell injury. *Biol Signals* **3**: 1-14, 1994.